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MEASUREMENT OF TRITIUM IN THE FREE WATER OF MILK

SPOTTING AND QUANTIFYING SOME BIASES AND PROPOSING WAYS OF IMPROVEMENT

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Abstract

As one of the three natural isotopes of hydrogen, tritium is ubiquitous and might potentially be present in any water or organic molecule that constitutes a biological matrix. Milk is one of the most frequently monitored foodstuffs in the vicinity of chronic release of radionuclides, as it is a very common product and also because it integrates deposition on large areas of grass at a local scale. Different parameters have been studied to assess their impact on the reliability of tritium measurements. The volume of the sample, the technique used to extract the water and the level of dehydration modulate the results but in different ways: dispersion of results, under- or overestimation of the tritium activity. The influence of sample storage and preparation has also been investigated. Methodological improvements of tritium measurements in the free water of milk are proposed.

Key words

Tritium measurement

Free water

Isotopic fractionation

Environment

Highlights

- Biases in tritium assay are caused by the conditions in which the water is extracted
- Isotopic fractionation does not fit with the Rayleigh formula when milk is distilled
- Recommendations are made to improve tritium activity measurement

Introduction

Among the unstable isotopes released by the nuclear industry, the quantities of tritium reaching the environment are usually small and generally fit easily with regulatory limits. As tritium was massively released during the atmospheric nuclear tests between 1945 and1980, it has become widely dispersed in the environment and in food chains. Its quantity in the atmosphere peaked in 1963 and has been decreasing ever since. It is now mainly localized in the water of oceans (about 99 %) (Jacobs, 1968; Weaver et al., 1969)(UNSCEAR, 2008). Nevertheless, tritium, along with ^{14}C and noble gases remain the dominant radionuclides released into the atmosphere by the nuclear industry. The main anthropic sources are weapon facilities, nuclear power plants, reprocessing facilities, the production and use of labelled compounds for medical use, research or even self-powered lighting products and research facilities for nuclear fusion (Guétat et al., 2008; IRSN/DEI, 2010).

Being an isotope of hydrogen, tritium can be incorporated into almost all components of biological systems: water (HTO) or organic molecules (Diabaté and Strack, 1993) (so-called Organically Bound Tritium or OBT). When dealing with OBT, two categories of atomic bonds are generally distinguished:

- binding to a nitrogen, oxygen or sulphur atom, i.e. labile bonds. It can easily be exchanged with labile hydrogen of other functional groups or molecules in its near vicinity, especially water; this fraction is called exchangeable Organically Bound Tritium (eOBT).
- binding to a carbon atom. Such covalent bounds are stable and therefore hydrogen atoms (or isotopes) are incorporated in the metabolic cycle of each molecule with more or less complex and lengthy features. This latter type of

bound tritium is known as non-exchangeable Organically Bound Tritium (neOBT).

Exposure of individuals depends on the type of the tritiated molecule(s) incorporated as well as on its/their metabolism. When tritium originates from tritiated water release and is further integrated in the food chain by, for example, going through photosynthesis (see further details in Boyer et al., 2009), some simplifications are considered to define a single "dose per unit intake factor" (ICRP 1989, 1997):

- considering exchangeable/non exchangeable proportions to be equal,
- considering an average biological half-life of 40 days for all non-exchangeable tritium of all organic molecules.

When using liquid scintillation counting, measurements of tritium specific activity on water allow the lowest decision threshold to be reached. Laboratories that measure tritium in environmental samples frequently use a same protocol:

- extraction of the free water of the sample and measure (Free Water Tritium), then
- on the one hand: oxidisation of the dry fraction resulting in the production of combustion water, then measurement of total organic tritium (i.e. the sum of eOBT and neOBT),
- on the other hand: isotopic exchange of hydrogen isotopes by washing the dry fraction with tritiated water, thus a second extraction of water to measure (if possible) eOBT and oxidisation of the "washed" and dried fraction to measure the neOBT as combustion water.

Frequently, eOBT is not measured but deduced from the following simple relation:

$$eOBT = OBT - neOBT \quad (1)$$

Every isotope or inaccuracy effect in every step of the procedure may induce errors in the measurement of the specific activity of extracted free water and of OBT (Baumgärtner and Kim, 1990; Kim and Baumgärtner, 1991).

Usually, water is extracted from fresh samples or after isotopic rinsing by at least one of the following four techniques:

- filtration: it allows quick and easy recovery of the main part of the dry matter, except soluble molecules which are in the filtrate. The bias induced depends on the filtration technique (i.e. characteristics of the filter) and on the nature of the sample. Retentate and distillate both need further treatment prior to measurement.
- distillation: it is performed under atmospheric pressure or under reduced pressure, it allows the recovery of almost pure water. Under reduced pressure, it is possible to completely distil at lower temperature (which induces less degradation of organic samples), to prevent the risk of contamination of extracted water by pyrolitic products (Wood et al., 1993) and to limit the isotopic effect during evaporation.
- azeotropic distillation extracts water at lower temperatures than distillation. As it uses organic compounds, it is more difficult to perform and it can additionally induce contamination of the dry matter by hydrocarbons.

- Freeze drying: i.e. extraction of water via sublimation; it has the same advantages as distillation under reduced pressure. The size of the apparatus, the temperature of the cold trap (usually > -20°C) and the time required to completely extract the water may induce biases by condensing atmospheric vapour before starting or during the process.

Repetition of measurements performed in our laboratory on the free water of a given tritiated milk obtained by distillation under reduced pressure or by freeze-drying, have shown certain systematic errors and dispersion of the values beyond the basic uncertainties of the measurements. The reasons underlying these differences have been sought and improvements of the reliability of tritium measurements are proposed.

Four possible hypothetical origins of the observed differences in measured specific activities were identified:

- the influence of sample storage: as ambient levels of tritium at the Valduc Centre of the French Atomic Agency can be higher than those of the environment where the samples were collected, they may become significantly more tritiated during their storage.
- the influence of the technique of water extraction: the usual techniques of dehydration differ from each other by their conditions of pressure, temperature and the apparatus used. These different factors may lead to biases in the measurement.
- the influence of the mass of sample: as each water removal technique has a specific dead volume and a specific geometry, the global yield of dehydration

can be influenced and thus be the origin of a bias in the measurement of specific activity.

- the influence of the final degree of dehydration: if isotopic fractionation occurs during water removal, the final level of dehydration will influence the specific activity measured.

1 Materials and Methods

Water was extracted from nineteen aliquots of the same milk sample (collected in the vicinity of the Valduc Centre of the French Atomic Agency) using three different dehydration techniques. The experiments were completed with twenty-one measurements performed on milks collected for our routine activity. Each time, weights of fresh milk, of dry matter and of collected water were noted.

1.1 Analytical method

Specific tritium activities were measured by liquid scintillation counting (PerkinElmer Tri-Carb 2910 TR) with an overall precision (2σ) of ± 17 %. The scintillator used was Ultimagold LLT (Packard). Quenching effects of the measuring system were carefully examined and the results measured corrected accordingly.

1.2 Storage of samples

The commercially available source water Volvic is considered to have very low levels of tritium. It is commonly used in laboratories as a blank. In order to check if storage of samples

in Valduc induced biases in the measurement of the specific activity, samples of Volvic water were stored in different conditions and their specific activity was been measured after 6 h, 16 h, 24 h, 48 h, 96 h, 1 week, 2 weeks, 3 weeks or 30 days of storage.

Modifications in the conditions of storage were performed to test the influence of the temperature and the type of bottle in which the samples were stored.

Four sets of nine samples of 50 mL of Volvic water were stored in 150 mL polyethylene bottles at -25°C, 3°C, 20°C and 40°C.

Five other sets of nine samples of Volvic were stored in different kind of bottles:

- 50 mL of Volvic water in 150 mL high density polyethylene (HDPE) bottles,
- 150 mL of Volvic water in the same kind of bottles,
- 50 mL of Volvic water in the same bottles placed in double welded vinyl bags,
- 20 mL of Volvic water in 20 mL glass bottles,
- 20 mL of Volvic water in 20 mL HDPE bottles (usually used for scintillation counting)

1 L of Volvic water was also stored in an open 1.5 L bottle. 10 mL were sampled after 6 h, 16 h, 24 h, 48 h, 96 h, 1 week and 2 weeks of storage. This experiment was shorter than the others since there was no water left in the bottle after the seventh sampling (due to evaporation and aliquot removal).

The results presented below are the means of four repetitions.

1.3 Techniques of water removal

1.3.1 Comparison of common techniques

Three commonly used techniques of dehydration were compared pairwise:

- distillation under reduced pressure using a distillation bridge with a Liebig condenser
- distillation under reduced pressure with a rotating evaporator (Buchi Rotavapor R200 or Buchi Rotavapor RE 121 equiped with Buchi 471 Oil bath)
- freeze drying using a Heto Drywinner PL3000.

Equal quantities of samples were used in each group of paired samples. In the first technique, the sample was introduced in a 1 L Erlenmeyer flask in a water bath at 55°C. The flask was fitted with a splash head (to prevent or limit the sample from spurting in the apparatus during distillation). The distillate was collected from a Liebig condenser containing a flow of 3°C thermostated water. Its dead volume was 0.69 mL ± 0.25 mL. The distillation bridge was connected to a Vacuubrand ME 2C pump working at full capacity. The condensate was recovered in an Erlenmeyer also at in a water bath at 3°C. At the end of dehydration, the first water bath was heated to 70 °C.

Distillation under reduced pressure was also conducted using rotating evaporator. This technique differs from the previous one by the apparatus used. One of the major differences between them is the geometry of the condenser: a rotating evaporator is equipped with a diagonal spiral condenser which has a dead volume of about 7 mL. Samples are introduced in a 1 L flask which is then connected to a rotating evaporator (Buchi Rotavapor R200 or Buchi

Rotavapor RE 121 equiped with Buchi 471 Oil bath) connected to a pump (Vacuubrand ME 2C) which is also used at its full capacity. Condensed vapours are recovered in a flat-bottomed flask. As with the previous technique, the water bath was thermostated first at 55°C and then at 70°C. The water flowing in the condenser was thermostated at 3°C.

Freeze drying was performed with a modified Heto Drywinner PL 3000. Samples were introduced in acrylic pots connected to a manifold which is connected to a glass insert. The insert was placed in the cold trap of the Heto Drywinner PL 3000 (temperature: - 55 °C) to allow the required decontamination of the cold trap between samples thus avoiding "memory effects". The manifold was also connected to the pump (Adixen Pascal 1005) used for general vacuum applications. Pressure in the system was < 0.5 hPa. After complete dehydration of the samples, the glass insert was removed from the system and immediately sealed to avoid contamination of the extracted water with atmospheric moisture until the ice has completely thawed.

Dehydration techniques were compared by pairwise to improve the power of the statistic tests (9 repetitions to compare freeze drying and rotating evaporator and 8 for freeze drying vs. distillation bridge and 8 for rotating evaporator vs. distillation bridge).

1.3.2 Test of the reliability of freeze drying and distillation

The specific activity of tritiated pure water (type 3 produced by RiOs 3 Water Purification System (Merck Millipore)) were measured in three cases:

- without other treatment,
- after being distilled under reduced pressure using a distillation bridge (as described above),
- after being freeze-dried (in the conditions described above). For each case, 3 aliquots of 49.9 g ± 0.1 g were prepared.

1.3.3 Influence of ambient atmosphere on freeze drying

To detect possible external contamination, two kinds of experiments were performed:

Measurement of the specific activity of water extracted by freeze drying in two different ambient atmospheres: one in the Valduc Centre (in the conditions described above) and one in Besançon (25-France) where the specific activity of the atmosphere in HTO is below the decision threshold. The milk was separated into 7 samples of 51.23 g ± 0.45 g. They were frozen in Valduc in plastic bottles inserted in double welded vinyl bags. Three were freeze-dried in Valduc, three in Besançon and one was distilled using a distillation bridge in the Valduc Centre in the conditions described above. Freeze drying at Besançon was performed in a Cosmos 20k (Cryotec). Vaccum was generated by a pump (Adixen Pascal 2005-Ci) working at full capacity. After the end of freeze drying, the water was recovered by heating the condenser. The specific activity of each recovered water sample was measured in the Valduc Centre and compared.

Empty freeze drying: Drywinner Heto PL 3000 was used empty three times for 5-7 days at Valduc Centre. A commercial bubbling system (MARC 7000-SDEC France) was used to monitor the atmospheric tritium levels during the third repetition. After the end of freeze drying, the mass of the cold trap was measured and compared to its mass when empty. Then, 10 mL of non-tritiated water was inserted into the cold trap to recover possible traces of water trapped during freeze drying. The specific activity of the water in the cold trap was measured, taking into account the dilution and compared to the specific activity of the water in the pots of the bubbling system.

1.4 Sample mass

To test the influence of the quantity of the sample on the reliability of measures, 6 masses were considered (about 15, 30, 60, 120, 240 and 480 g) and samples were treated by two of the three previously presented methods: distillation under reduced pressure using a distillation bridge or a rotating evaporator. Most of the sets were composed of two samples prepared with the distillation bridge and one with the rotating evaporator. The central point at 30-38 g was composed of three more samples treated with the rotating evaporator. The highest mass was only composed of one measurement since in other repetitions milk spurted throughout the apparatus until there was none left.

1.5 State of dehydration

The weight of fresh milk before treatment, and of dry matter and water after dehydration on the other hand, provide correlation between the mass of water extracted from milk and the specific activity measured.

"Sequential distillations" of milk were performed. The apparatus used in these experiments is illustrated in **Fig. 1**. A sample of about 300 mL was introduced in a 1 L Erlenmeyer flask in a 55°C bath. The flask was connected to a splash head to prevent or limit the sample from spurting into the apparatus during distillation. When the system is under reduced pressure, vapours flow to a condenser at 3°C and connected to a Vacuubrand ME 2C pump working at full capacity. The condensed vapour then falls into a dropping funnel. Each 10-30 mL (23 mL on average), it is opened to let the water flow into a 50 mL Erlenmeyer. Once the dropping funnel is empty, it is closed to collect the next aliquot and the water is collected from the 50 mL Erlenmeyer and weighed. The experiment is pursued until the sample is completely dry.

When possible, the dry matter of the sample is freeze-dried to collect any water which could remain. The specific activity of each aliquot is measured.

Fig. 1: Apparatus used for "sequential distillations”

Sequential distillations were carried out on four different milks collected for our routine measurements.

2 Results and discussion

2.1 Influence of samples storage

Only one set of samples showed any significant change in its specific activity during storage: the Volvic water stored in an open bottle (**Fig. 2**). The specific activity of the water increased until it reached equilibrium with the atmospheric water vapour (178.5 ± 133.0 Bq L^{-1}) after two weeks.

Fig. 2: Specific activity of Volvic water stored in an open bottle versus duration of storage. The first point having a specific activity under the limit of detection was plotted as having a specific activity of 0 Bq L^{-1}.

All the other results of the experiments remained below the decision threshold (2.8 Bq L^{-1}) during storage.

This proves that storage in well closed plastic or glass bottles is able to prevent the marking of the samples from the laboratory environment even in the case of the relatively tritiated atmosphere of a nuclear centre. Nevertheless, to avoid any cross contamination, we decided to store samples at -20°C (to preserve organic matter during storage) and in double packaging (bottle + sealed vinyl pocket or double sealed vinyl pocket) to avoid any unintentional marking.

2.2 Influence of the technique of dehydration

2.2.1 Comparison of three common techniques

The specific activity of water extracted from milks using the three techniques previously described was measured (mean: 60.4 g). The three techniques were not performed on each of the collected milk samples. **Fig. 3** shows how the results are distributed.

Fig. 3 : Comparison of the distributions of measured specific activities of water extracted using the three different methods described in this study. Central boxes represent the values from the lower to upper quartile. Middle lines represent the median. Vertical lines extend from the minimum to the maximum value of each population, which are represented by horizontal lines at their extremity. The specific activity of the water extracted by freeze drying is significantly higher than the specific activity of the water extracted by each of the two distillation methods tested (Wilcoxon test, $p < 0.01$). Differences between the two techniques of distillation were not significant.

On the one hand, both methods of distillation (distillation bridge and rotating evaporator) gave similar median concentrations but the rotating evaporator technique showed a standard deviation 22 % higher than the distillation bridge. On the other hand, the measured specific activities of water extracted by freeze drying were 40% higher when compared to the results obtained with a rotating evaporator.

Three experiments were performed to explain these results.

2.2.2 Reliability of distillation and freeze drying

The specific activity of pure water was measured with or without a complementary treatment (i.e. distillation or freeze drying) performed in the Valduc Centre. The results of this experiment are presented in **Fig. 4**.

Fig. 4 : Comparison of specific activities measured on water with and without treatment (distillation or freeze drying)

Distillation had no significant incidence on the measured specific activity of extracted water (+ 2.9 %) whereas freeze drying led to a clear increase (+ 42.9 %).

2.2.3 Influence of ambient atmosphere on freeze drying

First, to test the effect of ambient air during freeze drying, two sets of three 50 g-aliquots of the same milk sample were freeze-dried, one set in Besançon and one in the Valduc Centre. Freeze drying in both the Valduc Centre and Besançon led to almost complete extraction of the water, i.e. 88% of the weight of the total sample without any significant difference between samples (standard deviation: 0.09 %). Nevertheless, the comparison of the specific activities of the two sets of samples did show significant differences (see **Fig. 5**).

Fig. 5 : Specific activities of water extracted from milk by means of freeze drying performed in Besançon and in the Valduc Centre.

This difference can only be explained by a (de)marking of the extracted water by the condensation of atmospheric water vapour. This phenomenon can occur during freeze drying by leaks in the apparatus, or before freeze drying by condensation of atmospheric water on the frozen sample or even after freeze drying when the vacuum is broken to recover the condensed water. Note that the observed deviation after freeze drying in Besançon is high regarding the little difference of specific activities between atmospheric water (< 5.8 Bq L^{-1}) and free water of milk (about 20 Bq L^{-1}).

The apparatus used in the Valduc Centre avoided the risk of exchange during melting of frozen condensate but this was not the case in Besançon. This can explain why the impact of condensed atmospheric vapour was so significant in Besançon whereas the differences in

specific activities remained small. In laboratories specifically equipped for measurement of tritium (for example with a small cold trap which can be isolated from the atmosphere while the condensed water is recovered), the biases would be, at worst, in the measurement uncertainty interval.

Secondly, empty freeze dryings were run in the Valduc Centre. They showed recovery of water with a significant specific activity. The results are presented in Table 1.

Table 1: Mass and specific activity of water recovered after empty freeze dryings performed in the Valduc Centre

Duration of freeze drying (h)	Mass of recovered water (g)	Specific activity of the recovered water (Bq L-1)
168	0.4	200
168	0.0	No measurement
146	0.8	257

The results of the first and third freeze dryings fit well with the results presented in **Fig. 4**. For example, 0.8 mL of "parasite" water with a specific activity of 257 Bq L^{-1} can explain an overestimation of the specific activity of about + 4 Bq L^{-1} in a sample of 47.5 mL in which the specific activity is about 23 Bq L^{-1}.

Nevertheless, the mass of water collected during freeze drying appears variable, as does its specific activity. This indicates that an intermittent mechanism (most probably depending on atmospheric conditions) leads to the pollution phenomenon.

In the third repetition, the specific activity of the recovered water was about 257 Bq L^{-1} where the specific activity of atmospheric vapour measured by bubbling during freeze drying was only 90.8 Bq L^{-1}. This means in our opinion that, in addition to a possible leak in the apparatus (before the cold trap), four other ways of contamination might be suspected:

- as the sample is frozen before freeze drying, atmospheric water is able to condense at its surface in the time lapse between storage and introduction into the freeze drying system;
- the water vapour in the air present in the freeze drying system (about 6 L in the case of the Heto Drywinner PL 3000) is prone to condense starting from the moment when the cold trap is at its set-point temperature to the instant when vacuum is established in the system;
- the water vapour in air that fills the freeze drying system when the vacuum is broken at the end of freeze drying can also condense in the cold trap;
- some exchanges may occur from the atmosphere to the condensed water while the water melts in the cold trap for recovery.

It appears that during the different steps of a freeze drying process, some atmospheric water can be condensed or exchange and therefore be mixed to the extracted free water of the samples. The efficiency measured in water recovery is of the order of 97 %. The mass of external water cannot be identified in the different tests because it surely compensates sample water which is lost at the same time. This phenomenon was fortunately discovered due to the ambiance in the Valduc Centre that is sufficiently tritiated to be measured. Nevertheless, marking of extracted water during freeze drying may occur in other laboratories with very low

tritiated atmosphere. In that case, the water extracted would be “demarked” with non-tritiated water, even if it is within the uncertainty interval of measure.

One way to limit the deviation due to this pollution of the extracted water would be to freeze dry larger samples to dilute the effect in the water extracted from the sample or to operate freeze drying in a dry atmosphere.

2.3 Influence of the mass of sample in distillation techniques

The free water of nineteen aliquots from the same milk sample was extracted by distillation using a rotating evaporator or a distillation bridge. The results of this series of experiments are presented in **Fig. 6**. As expected, the nineteen specific activities measured are well described by a Gaussian distribution. Uncertainties ranged from 14 % for higher specific activities to 17 % for lower specific activities.

Fig. 6: Specific activity of extracted water as a function of the mass of treated samples and normality of the distribution of density of measured specific activities

Small samples (< 60 g) and large samples (≥60 g) present almost the same average value, respectively 22.3 Bq L^{-1} and 22.8 Bq L^{-1}, but different standard deviations: 2.17 Bq L^{-1} and 0.88 Bq L^{-1}. The variation of these average values may be explained by differences in the proportions of water extracted in each case (respectively 84.4% vs. 85.9 %).

Treating very large samples (> 100 g) is not easy: experimentally we observe that the milk is boiling and spurting very rapidly after the beginning of each repetition. This phenomenon is prone to contaminate both extracted and condensed water. Treating small samples allowed the

use of larger vessels compared to the sample volume: a difference of a factor of 5 between the volumes of the Erlenmeyer flask and the sample is sufficient to limit spurting.

Each distillation technique also showed its own limitations with regard to the mass of the samples treated.

Fig. 7: Proportion of water extracted (in % of weight of total sample) versus mass of sample treated. The mean proportion of free water obtained was evaluated by measuring the dry mass of each sample remaining after 5 h in a forced-air oven at 102°C.

While the rotating evaporator efficiently dehydrates only samples between 100 g and 400 g, the distillation bridge is efficient for a larger range of sample volumes (only one dehydration was not complete as it was interrupted to soon) (Fig. 7).

In these experiments, distillations of 60-100 g milk samples under reduced pressure using a distillation bridge gave the best results with a limited dispersion of the measured specific activities.

2.4 Influence of the state of dehydration

The specific activities measured were viewed with respect to the state in which each experiment was ended. The results are presented in Fig. 8. The specific activity appears to increase slightly with the degree of dehydration but neither Spearman's nor Student's correlation tests revealed a correlation between the two parameters.

Fig. 8 : Specific activity of extracted water versus the degree of dehydration

The line shows the same relationship modelled by relation (10) with $p = 0.04$, $\alpha = 1.14$, and $\beta = 11.7$.

Further experiments were then carried out with sequential distillations performed on different milks. The results are shown in Fig. 9 and Fig. 10. To facilitate comparison of the results, specific activities are expressed as relative activities (1 corresponds to the mean specific activity at the end of each sequential distillation) and levels of water extraction are expressed as a % of the total mass of sample at the end of each distillation. In Fig. 9 the specific activity of each aliquot seems to follow a two-component function.

Fig. 9 : Specific activity of aliquots of extracted water versus the proportion of water extracted
The line plots the variation modelled with equation (11) with $p = 0.04$, $\alpha = 1.14$, and $\beta = 11.7$. Each type of label represents a set of repetition. Four repetitions were performed on one sample of milk (squares), two on a second sample (triangles) and lozenges represent a set performed on a third sample. Mean specific activity of extracted water = 1.

Fig. 10 : Mean specific activity versus proportion of water extracted. The solid line was computed using relation (10) with $p = 0.04$, $\alpha = 1.14$, and $\beta = 11.7$. Each type of label represents a repetition set. Four repetitions were performed on one sample of milk (squares), two on a second (triangles) and lozenges represent a set performed on a third. Mean specific activity of extracted water = 1

Isotopic fractionation during distillation is generally described by means of the Rayleigh equation that was first derived for fractional distillation of mixed liquids (Rayleigh and Strutt, 1902).

(2)

where:

n_0: is the initial number of all moles of all species in the sample

n: is the number of all moles of all species in the residual sample

X_0: is the initial mole fraction of HTO in the sample

X: is the mole fraction of HTO in the residual sample

α: is the vapour-liquid fractionation factor

Kim and Baumgärtner (1997) reported that tritium enrichment on distillation of pure HTO/H_2O can be calculated using the Rayleigh formula taken under the following form (3):

$$A_r V_r = A_0 V_0 \left(\frac{V_0}{V_r}\right)^{\frac{1}{\alpha}} \quad (3)$$

Where:

A_r : is the specific activity of residual water

A_0 : is the initial specific activity of the water sample

V_r: is the volume of residual water

V_0: is the initial volume of water in the sample

α: is the vapour-liquid fractionation factor

Under equilibrium vaporization conditions, α may be equivalent to the vapour pressure isotope effect (VPIE) that can be calculated theoretically (Van Hook, 1968) or determined experimentally (Baumgärtner and Kim, 1990) under given approximations. The VPIE corresponds to the definition provided in (4) and is considered equivalent to the separation factor ignoring the corrections accounting for both a non-ideal liquid and gas phases (Jancso and Van Hook, 1974; Kakiuchi, 2000):

$$VPIE = \frac{P_{H_2O}}{P_{HTO}} \approx \alpha = \frac{(X_T / X_H)_L}{(X_T / X_H)_V} \quad (4)$$

514 P_{H2O} and P_{HTO} are the vapour pressures of pure water and pure tritiated water, respectively,
515 X_T and X_H stand respectively for the molar fractions of HTO and water, in the liquid (L) and
516 vapour (V) phase.
517
518 In order to fit with our experiments, residual volume (V_r) and specific activity (A_r) were
519 replaced in (3) by extracted volume (V_e) and specific activity (A_e) using relations (5) and (6).
520 Equation (7) is thus obtained:

521 $$V_e = V_0 - V_r \quad (5)$$

522 $$V_e A_e = A_0 V_0 - A_r V_r \quad (6)$$

523
524 By considering (3):

525 $$V_e A_e = A_0 V_0 \left(1 - \left(\frac{V_0}{(V_0 - V_e)} \right)^{\frac{-1}{\alpha}} \right) \quad (7)$$

526 Lastly, each aliquot sampled during the distillation has a specific activity ($A_{(1-2)}$) which is the
527 mean value of (7) between V_1 and V_2, respectively the volume of water extracted at the
528 beginning and at the end of the extraction of the given aliquot (8):
529

530  (8)

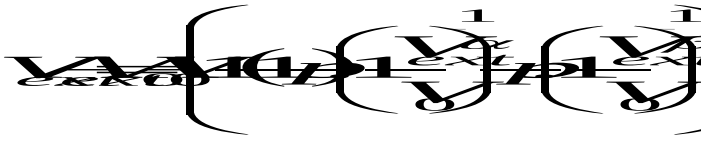
531
532 Equation (8) fits experimental data provided by distillation of pure HTO/H_2O (Fig. 11). In
533 these experiments, α was evaluated at 1.14 which is 6.7 % higher than expected in our
534 experimental conditions (Baumgärtner and Kim, 1990). This difference may be explained by
535 the specificities of the apparatus used which seem to increase the height by the equivalent of a
536 theoretical plate (HETP) and thus α (Fukada, 2004).

537

538 **Fig. 11 :** Extraction of HTO during distillation of pure HTO/H_2O versus proportion of free water extracted
539 Lines are calculated by equations (7) and (9). Triangles and circles correspond to experimental values. Mean
540 specific activity of extracted water = 1
541

542 Conversely, with samples of milk, at percentages of free water extracted higher than ~ 95 %,
543 (7) and (8) generally fail at modelling the observed experimental behaviour of the relative
544 activity of extracted water, due to the drastic increase in relative activity measured at the end
545 of the water extraction process (Fig. 9). When most of the water is extracted from the milk,
546 the remaining part of the sample in the boiler is likely to behave as a non-ideal solution. It is
547 thus necessary to introduce a correction in (7) and thus in (8). Such a correction may be
548 obtained in two main ways: a complete theoretical description of the sources of non-ideality
549 or a blind parameterization of the observed effect. As for the theoretical description, in its
550 simplest form a model may at least take account of two kinds of water, cosphere (hydration)
551 water in the immediate neighbourhood of solute particles or molecules and bulk water which
552 retains the properties of the pure solvent (Jancso and Van Hook, 1974). In this part of the
553 study, we will try to parameterize the observed effect and confine our work to the
554 consequences of this behaviour on the metrology of tritium specific activity.
555

556 To take account of the drastic increase observed at the end of the water extraction, equation
557 (7) is parameterized by introducing two dimensionless free parameters p and β in the
558 following manner:

559  (9)

560 The specific activity of an aliquot is thus given by (10):

561  (10)

The experimental data presented in Fig. 9 can be modelled using relation (10). The best values for p, α, and β (which are respectively 0.04, 1.14, 11.7) were estimated by a function in the R software (R Core Team, 2012) which carries out minimization of a function (f) using a Newton-type algorithm. In R software, this function is called nlm. Each first aliquot of the different repetitions has a variable specific activity as compared to the mean final specific activity of the set. This is most probably an artefact linked to the experimental conditions. It is noticeable in Fig. 10 that this value has a perceptible impact on the mean specific activity of, say the 4-5 first aliquots. Using the model based on relation (9) we learn that even with a (hypothetically) perfect dehydration apparatus, if the dehydration is interrupted when 10% of water remains (a situation that may happen if the temperature is too low, the pressure too high or the dehydration simply is uncompleted); the measured specific activity of the extracted water should thus be underestimated by about 9 %.

2.5 The dead volume, an example of a combination of sources of biases

In light samples (ranging from 15 g to 60 g), the measured specific activities of water extracted with a rotating evaporator were systematically lower than those obtained with a distillation bridge. Additional experiments were performed to explain this particular point. The rotating evaporator condenser indeed has a dead volume estimated to be 7.0 mL ± 1.2 mL. In other words, 7 mL must reach the condenser before the first drop of distillate is observed and 7 mL remain in the condenser at the end of distillation. A model of the time-course of the specific activities in the condenser and in the distillate during distillation based on an isotopic fractionation was set up.

Let us now consider the following assumptions as axiomatic:

- as described above isotopic fractionation exists,
- in the condenser of the rotating evaporator, equilibrium between vapour and condensed phase is instantaneous,
- each mass of vapour that flows up from the boiler to the condenser gets rid of the same mass of condensed vapour to the distillate.

Now, introducing the subscript “cond.” to denote the activity (or the mass) in the condenser; from the beginning of the distillation to the extraction of a mass of water equal to the mass of water in the dead volume, we have:

$$A_{cond} = A_{ext} \tag{11}$$

As soon as the dead volume of the condenser is filled:

$$A_{cond} = \frac{V_{cond} A_{cond} + V_{dist} A_{dist}}{V_{cond} + V_{dist}} \tag{12}$$

We consider that no water flows into the distillate until the dead volume of the condenser is full and introduce the subscript “dist.” to denote a specific activity of the distillate, thus:

$$A_{dist_i} = \frac{V_{cond} A_{cond} + V_{dist} A_{dist}}{V_{dist}} \tag{13}$$

This model was tested on the different data sets of our results as well as with an experiment where the condenser was first saturated with untritiated water before distilling tritiated water. All sets of results are well-fitted by the present model. It shows how the measured specific

activity (measured in the distillate) is modified by the loss of water in the dead volume (Fig. 12) for a known dead volume (7 mL in this example).

Fig. 12: Modelled evolution of specific activity of water in different compartments during the distillation of a 20 g sample of milk (Mean specific activity of extracted water = 1).

This influence depends on the mass of the sample and on its specific activity (Fig. 13). For samples of milk lower than 10 g, the bias is less than 3 %. Actually, as there is only 8.7 g of water in 10 g of milk and the dead volume of the condenser being estimated to 7 mL (7 g), the few drops that flow out from the condenser has a specific activity that is fully representative of water extracted from the sample. For samples with $15\ g \leq M \leq 75\ g$, the underestimation of the specific activity is about - 6 %. The latter is in good agreement with the results presented in Fig. 8 which represents how specific activities of different samples are distributed as a function of their final rate of dehydration. Lastly, using samples larger than 200 g is a necessary condition to obtain a deviation that remains below 3 % when using a rotating evaporator.

Fig. 13: Bias due to the rotating evaporator versus mass of sample. The model described above was tested with different masses of sample (from 7 g to 10 000 g) to determine how the modelled bias induced by the dead volume of the condenser $\left(\frac{A_{measured}}{A_{theoretical}}\right)$ evolves in this range of mass.

3 Conclusion

In this study we show that each methodological aspect tested (water removal technique, mass of sample and final state of dehydration) is able to induce a bias in the specific activity measured in the extracted water. In most environmental monitoring situations, these biases

638 remain close to the uncertainty of measurement when liquid scintillation is used in the usual
639 conditions (i.e. about 15% when measuring a sample of 10 mL with an activity of 20 $Bq.L^{-1}$
640 water mixed with 10 mL of UltimaGold LLT (Pointurier et al., 2003) for 200 minutes).
641 Nevertheless, some of these biases can produce systematic underestimations of the actual
642 specific activity. First, it was shown that in the conditions of this study the water extracted by
643 freeze drying had a specific activity significantly higher than the water extracted using a
644 distillation technique based on the same milk sample. This is explained by a pollution of the
645 extracted water by atmospheric water before, after or during the freeze drying process. This
646 particular behaviour became apparent due to the fact that atmospheric water in the Valduc
647 centre is has slightly elevated ambient levels of tritium. Caution must thus be taken to avoid
648 marking during freeze drying, especially when the specific activity of the sample is not of the
649 same order of magnitude as the specific activity of the atmospheric vapour. An easy and
650 economic way to prevent this phenomenon would be to freeze dry larger samples (at least 100
651 g) which would dilute the effect of atmospheric water condensation. A more suitable way
652 nevertheless would be to operate freeze drying in a dry atmosphere and to break the vacuum
653 with dry gas.
654
655 When dehydration is performed by distillation under reduced pressure, the dead volumes of
656 the devices (especially those of the condenser) have to be limited as they can induce a bias in
657 the estimation of the mass of extracted water as well as and in the measurement of specific
658 activities. It appears that using a distillation bridge suits a wider range of samples masses than
659 a rotating evaporator, the latter should be preferred for large samples (> 300 mL).
660
661 Lastly, in this study a fractionation effect during dehydration proved to be more significant
662 than expected. This shows the necessity to perform dehydration until there is no water left in

663 the sample. When a fraction of water cannot be extracted without taking the risk of damaging
664 the dry matter, the residual fraction of water should be estimated and the specific activity
665 measured corrected using formula (9) proposed in this work.
666
667 The effects of the different sources of biases must be summed. For instance, if a distillation
668 using a rotating evaporator (dead volume of 7 mL) of a sample of 20 mL of milk is
669 interrupted when 10 % of water remains in the matrix, the measured specific activity would
670 be about 91 % of the real specific activity and the standard deviation of this result would be
671 11 % additionally increase by the uncertainty of measure (about 15 % in usual conditions).
672 Overall, this would lead to a global underestimation of about 10 % and an uncertainty of ± 26
673 %.
674
675 In the literature, VPIE has been shown to decrease while temperature increases without
676 differences being measured between vaporization and sublimation (Baumgärtner and Kim,
677 1990). It has also been shown that a link exists between isotopic fractionation during
678 extraction of water and a three-layer model for bound water (Kim and Baumgärtner, 1997)
679 (described by Drost-Hansen). The results gathered herein using milk samples show
680 fractionation behaviour which can be described by a two-component formula, each
681 component being based on specific Rayleigh distillation processes. These results bring to
682 mind the features of two fractions of water that coexist in milk: the first one (96%) that acts as
683 pure water (free water) and the second (4%) which presents an isotopic separation factor β
684 equal to 11.7 much higher than that of pure water; $\alpha = 1.14$. The fraction described here by
685 the isotope separation factor β may be bound water.
686
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